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THE STOMACH SPIROCHETE OCCURRING IN MAMMALS

KATSUYA KASAI AND ROKUZO KOBAYASHI

From the Laboratories of the Kitasato Institute for Infectious Diseases, Tokyo

In 1893 Bizzozero discovered a spirochete in the stomach of dogs in Italy which he stated was in the parietal cells of the peptic glands. Salomon (1896), examining various animals in Germany, detected the same organism in the stomach of dogs, cats and rats, and was able to transmit it by feeding to the stomach of the mouse. He distinguished three forms morphologically and also found the spirochete to be actively motile with terminal flagella. Balfour (1906), in Egypt, observed spirochetes in gastric and intestinal ulcers of dogs and monkeys, produced by inoculation with a trypanosome (*Trypanosoma dimorphon?*). In the same year Krienitz (1906), in Germany, found three forms of spirochetes in the fresh stomach contents of a patient suffering from stomach cancer and later (1906a) studied the morphological changes resulting from alternations in environment. Afterward Regaud (1909), in France, found the organism by means of darkfield illumination and proved it to be a living micro-organism, notwithstanding that Carnot and Lelièvre (1909) had described it as the secretion product of parietal cells. In the following year Lucet (1910) detected two forms of spirochete in lesions of a dog suffering from hemorrhagic gastro-enteritis. Ball and Roquet (1911), however, regarded this spirochete as identical with that described by Regaud and called it *Spirochaeta regaudi.* They stated, moreover, that this spirochete occurring in the normal stomach of dogs has probably no causative relation to hemorrhagic gastro-enteritis. Suda (1916), in Japan, also observed spirochetes in the gastric gland of dogs.

The present paper is a report of investigations undertaken in connection with our encountering this spirochete in the stomach of a rat in the course of an examination of the alimentary tract (1917).

Morphology.—The stomach spirochete is an inflexible spiral with a straight longitudinal axis, the transverse section being an ellipse, and is provided with one short, thin flagellum at each end. In the stained preparation, the most common type of this spirochete (Fig. 1) is a fine form measuring from 4.5 to 10.5μ and the number of turns is 4 or 5 to

14 or 15. Every two consecutive turns are closely set, the distances between these pairs of spirals being regular. There was frequently seen, however, in the stomach of the cat and monkey a form somewhat shorter, with a wider spiral and a more acute turn (Fig. 2). Another form (Fig. 3), usually occurring in the stomach of the dog, is stout, and the turns are shallower and fewer. Such a form is especially observed in the stomach of the rabbit, and also often of the mouse and guinea-pig, if it be transmitted.

Thus, like Salomon, we were able to distinguish three forms morphologically, although our classification is slightly different. It is uncertain whether these forms represent in reality three different species or belong to one species with temporary variations in form. The degenerated spirochete frequently assumes the spiral body curved or twisted, or with irregularly relaxed turns. There are also seen individuals faintly or heterogeneously stained, and even in "moniliform degeneration" (Fig. 1). Moreover, in preparations stained with Giemsa's stain, we can very often detect so-called "involution forms," which show from one to five or more chromatin-like granules stained intensely red at the outer ends of turns, notwithstanding the faint staining of the body. Such granules also are occasionally detected arranged along the main axis in the rod-shaped spirochete (Fig. 3).

This spirochete in the supravital staining is not found differentiated from that of the foregoing description.

The spirochete in the dark-field illumination shows a fair distinction (Fig. 4). The consecutive turns seem almost to touch, and accordingly the whole body presents the appearance of a coil, the transverse section of which was clearly proved by the rotatory movement of the organism to be an ellipse. The dark-field microscopic view also shows that each of the two extremities of this spirochete is tapered into a fine terminal flagellum.

Staining.—Compared with the other spirochetes, the spirochete under discussion takes stain very readily with the basic anilin dyes, viz., fuchsin, methylene blue, gentian violet, etc. For the staining of smears, however, Manson's borax methylene blue, Giemsa's stain and Fontana-Tribondeau's method are especially to be recommended. For the staining of the organisms in tissues, iron-hematoxylin staining is superior to Levaditi's silver impregnation. Here Levaditi's method, although it is convenient for proving the existence of terminal flagella, is not suitable for differentiating the spirochetes in the stomach glands (Fig. 5). This is probably due to the mucus, which surrounds the organism and perhaps prevents its impregnation with silver.

As the relief staining, Benians' (1916) method is not only simple, but gives an excellent preparation (Fig. 6). The procedure is as follows: A small drop of a 2 per cent. aqueous solution of Congo red

is placed on a slide and a very small quantity of the material to be examined is rubbed into it with the platinum loop. The drop is then spread out into a rather thick film and allowed to dry. The slide is then washed over with a 1 per cent. solution of HCl in absolute alcohol and dried in the air. The film is then ready for examination.

Movement.—The movement of this spirochete is comparatively rapid and very simple. Examination under the dark-field microscope shows that the organism moves only forward and backward inflexibly in a straight line, and progression always takes place by the vibration of the posterior flagellum. Occasionally, however, there were observed a rotatory movement around the long axis, a snakelike movement, a movement forming the outline of a cone with a fixed end as apex, etc.

It must be remembered that, for examination by dark-field illumination, the material to be examined must, in most cases, be diluted with a few drops of water, otherwise the free movement of the organism is decidedly limited by compression of the tissue mass.

Distribution Among Animals.—We examined the stomachs of thirteen monkeys, forty-nine dogs, thirteen cats, twenty rabbits, fifteen guinea-pigs, thirty-eight wild rats, ten white rats, fifteen mice and fifteen field voles. All of the specimens examined were fresh, the animals having been killed only a short time before examination, except in the cases of six dead dogs and four cats, which, however, were examined shortly after death. The result of our examination is as follows:

1. The spirochete was detected in forty-three out of forty-nine dogs. Five out of six negative cases, however, were young from the same mother, only two or three weeks after weaning.

2. Out of thirteen cats eight gave a positive result, and the five negative cases were all very young.

3. Thirty-eight wild rats yielded only one positive case (*Epimys rattus alexandrinus*).

4. Thirteen monkeys were all positive.

5. Among rabbits (inoculated with *virus fixe* of rabies), guinea-pigs, white rats, mice and field voles, there was no positive case.

Judging by the foregoing results, the invasion of this spirochete seems to have a close relation to the life condition of the host. It was detected in nearly all cases of adult dogs and cats, which wander from place to place, devouring whatever food they happen to find. One hundred per cent. of the monkeys in which the spirochete is found show an extensive variation of diet. The limited life, on the other hand, may explain the rare occurrence of this organism among young dogs, young cats and wild rats, and its non-occurrence among other experimental animals, such as white rats and mice.

Distribution in the Animal Body.—We looked for the spirochete in various parts of the alimentary canals of twenty-six animals, naturally or experimentally infected; i. e., five dogs, two cats, three wild rats, five white rats, six mice and five rabbits. While the organism was always detected abundantly in the stomach of all these, in the mouth cavity and cecum, no similar spirochete could be found in any animal. But a few degenerated specimens were detected in the esophagus of four dogs, one cat, one wild rat, one white rat, one mouse and three rabbits, and still fewer in the duodenum of one dog, one white rat and one mouse. Moreover, the stomach contents were examined in four dogs, one cat, two white rats, one wild rat, one mouse and five rabbits, and only a few degenerated specimens were detected there in three dogs, one white rat and three rabbits.

These experiments indicate that the domicile of this spirochete is the stomach. Histological examination also shows that it is principally detected in the fundus gland, especially in its neck, where the organisms arranged parallel to the axis of the duct occasionally swarm so densely as to obstruct the canal. Moreover, organisms were seen lying between the chief cells or even in the cytoplasm of the parietal cells. In the case of dogs and cats, spirochetes were eventually found in the pyloric gland.

Transmission Experiment.—Mice, white rats, guinea-pigs and rabbits were selected as experimental animals. In this experiment, about five mice, two or three white rats, two or three guinea-pigs, and usually two rabbits were used for transmission from generation to generation. Here the canine strain was principally used, but occasionally the feline or the monkey strain was employed. These strains gave almost the same result.

To transplant the original strain to experimental animals of the first generation, we scraped the mucous membrane containing large quantities of this spirochete from the stomach of a dog, and fed to mice and rats in small portions and to guinea-pigs and rabbits in large amounts. After the second generation, in the case of mice and rats, a piece of the stomach wall was given to each animal of subsequent generations, and guinea-pigs and rabbits were fed a large quantity of the finely crushed mucous membrane.

Following are the results of the transmission experiment:

1. In the case of mice, we obtained the most satisfactory result, distinct multiplication being observed as early as the second day after transmission. The procedure was continued for fifteen generations, and it was found that there was a remarkable increase in every generation.

2. The transmission was also very easy in the case of white rats and the experiment was therefore discontinued after the tenth genera-

tion. The same result was obtained in the case of wild rats, where passage was continued until the fifth generation.

3. In the case of normal guinea-pigs, the transmission was very difficult. The first experiment was continued with difficulty until the third generation; by making use of animals infected by scarlet fever or measles, however, we were easily able to continue it until the tenth generation.

4. In the case of normal rabbits, we were unable to carry the procedure through the fifth generation. If the animal infected with this spirochete be inoculated with *virus fixe* of rabies, however, transmission becomes extraordinarily easy. The canine strain was thus passed without difficulty through ten generations and the feline through five generations.

Experiments on Resistance

I. Lytic action of saponin, sodium taurocholate and bile

The material used for the experiment was the feline strain. The mucous membrane, in which large numbers of spirochetes had been detected, was scraped from the stomach of a cat immediately after death, and diluted with saline solution. Saponin and sodium taurocholate were used in 10 per cent. aqueous solution. The bile was obtained from the cat and used without being diluted.

The procedure was as follows: Equal quantities of each of these chemicals and the spirochete-containing suspension were thoroughly mixed and, at required intervals, a small drop of it was spread upon a slide by means of platinum loop. It was then dried above a weak flame (about two minutes), fixed with methyl alcohol for fifteen minutes and stained by Manson's stain under as nearly the same conditions as possible.

The result of this experiment is recorded in Table 1.

TABLE 1.—LYTIC ACTIONS OF SAPONIN, SODIUM TAUROCHOLATE AND BILE ON THE SPIROCHETE

Chemicals	15 Mins.	30 Mins.	1 Hr.	2 Hrs.	3 Hrs.
Saponin	Spirochetes became swelled and stained unfavorably. Some in process of dissolution	Staining very faint, and spirals irregular and indistinct	Almost complete dissolution	Almost complete dissolution	A very few degenerated organisms remaining
Sodium taurocholate	A few degenerated spirochetes remaining	Complete dissolution			
Bile	A few spirochetes in process of dissolution	Degenerated forms unlike the spirochete seen very rarely	Complete dissolution		
Control	No change	Degenerated forms unlike the spirochete seen very rarely	Complete dissolution	Almost complete dissolution	A very few degenerated organisms remaining

II. RESISTANCE OF THE SPIROCHETE AGAINST PUTREFACTION

(a) *Experiment in the Refrigerator.*—This experiment was made in July and August. The contents having been removed, the stomach wall containing spirochetes was placed in the refrigerator (8 to 10° C.). Every other day some of the mucous membrane was scraped off and fed to two mice, to ascertain whether it still contained live spirochetes. In three specimens from the dog and in two from the cat the results indicated that the spirochete under discussion generally continues its life in such condition for about ten days (from seven to fourteen days), showing that the death of this organism has a close relation to the putrefaction of the stomach wall. If after ten days the stomach wall putrefies to any degree and spirochetes are no longer perceived under the microscope, transmission to mice generally becomes impossible.

(b) *Experiment in a Room.*—This experiment was performed in August. The materials obtained from one dog and three mice, all heavily infected, were exposed in a room (average 30° C. in the former case and 28° C. in the latter) for twenty-four hours and the putrefied material given to two mice. Except in the case of one mouse, the result was negative. If the contents be allowed to remain in the stomach, however, this spirochete seems to disappear more quickly. We observed that the spirochete in question disappeared within about ten hours in such a stomach, even in the refrigerator. The result is probably due to the lytic action of split products of the stomach contents.

III. ACTION OF SALVARSAN ON THE SPIROCHETE IN VIVO

(a) *Infusion of Salvarsan Into the Infected Stomach.*—We selected as the experimental animals mice previously infected with large numbers of spirochetes. Arsaminol (salvarsan made in Japan) was used as an acid solution diluted only with saline solution, viz., 1 : 100, 1 : 200, 1 : 300, 1 : 500, 1 : 1,000 and 1 : 2,000. The solution was introduced directly into the stomach cavity of the mouse (1 c.c. per cap.) by means of catheter at the time of starvation. Twenty-four hours later the mice were killed and the mucous membrane of the stomachs examined under the dark-field microscope.

The result is indicated in the following table:

TABLE 2.—STERILIZATION EFFECTS OF ACID SALVARSAN SOLUTION ON THE SPIROCHETE IN VIVO

Mouse Number	Degree of Dilution of Salvarsan	Spirochete
1, 2, 3	1 : 100	—
4, 5, 6	1 : 200	—
7, 8, 9	1 : 300	—
10, 11	1 : 500	+
12, 13	1 : 1000	+
14, 15	1 : 2000	+
16, 17, 18, 19	Control	+

N.B.—Mouse 9 died about 15 minutes after infusion. Examination of the stomach revealed no spirochetes.

The table shows that the 1 : 300 acid solution of salvarsan can still sterilize the spirochete in the stomach of the mouse.

(b) *Intravenous Injection of Neosalvarsan.*—The 1 : 200 solution of neoarsaminol (neosalvarsan made in Japan) was intravenously injected into the five spirochete-bearing mice to the amount of 0.05 c.c. for 1 g. of weight, viz., in the maximum dose. Thirty hours later the mice were killed and their stomachs examined by dark-field illumination. The stomachs showed the same negative result as the controls (two normal mice).

Pathogenicity.—If the host is normal, occurrence of this spirochete in the stomach exerts no pathogenicity. If the spirochete-bearing rabbit be inoculated with the *virus fixe* of rabies, however, the spirochete abundantly increases in number and causes a specific lesion in the stomach of the host.

Such rabbits, showing rabid symptoms a week after the inoculation of the *virus fixe,* were killed, and on examination the stomach usually contained only fluid with no food particles. A large quantity of mucus always covered the surface of the mucous membrane. Marked hyperemia and hypertrophy of the mucosa, especially in the fundus, were also present. In such cases, *punctate hemorrhages, even the so-called hemorrhagic erosions, were constantly detected on both sides near the middle along the greater curvature.* Upon histological examination, the hyperemic and hemorrhagic areas were found to be located principally in the mucosa, especially in the free end of the glandular layer, but frequently also in the submucosa. The spirochetes were always abundantly detected in the lesions, where they appeared not only in the ducts of glands but sometimes even in the tissue, while they were rarely, if ever, found in the apparently normal parts. No such remarkable lesion has ever been seen in the stomach of the rabbits infected only with the *virus fixe.*

Tables 3 and 4 show the results obtained with the canine and feline strains. They also show that if, a certain interval after infection of this spirochete, the rabbit be inoculated with the *virus fixe,* the autopsy performed one week later will show that abundant increase of the spirochetes causes a severe hemorrhagic gastritis in the host. It is concluded that the infection with the *virus fixe* probably causes a gastric disturbance apparently as invisible as a very slight catarrhalic gastritis in the rabbit, and that a stomach so affected becomes a favorable medium for this spirochete. Then large increase in the number of spirochetes seems to cause the slight primary disturbance secondary to the heavy hemorrhagic gastritis. The reason for this secondary pathogenicity, however, is still uncertain. The same experiment was repeated on ten mice, and only three cases of the slight hemorrhagic gastritis were detected.

TABLE 3.—RESULT OBTAINED BY THE INOCULATION OF THE VIRUS FIXE INTO THE CANINE-STRAIN BEARING RABBITS

Rabbit No.	Generation	Date of Transmission of Spirochetes	Date of Inoculation of the Virus Fixe	Interval between Transmission and Inoculation	Occurrence of Spirochetes	Lesion of the Gastric Mucosa
1	I	May 28, 1917	May 28, 1917	0	++	—
2	I	May 28, 1917	May 28, 1917	0	—	—
3	I	May 28, 1917	May 28, 1917	0	—	—
4	I	May 28, 1917	May 29, 1917	1	—	—
5	II	June 4, 1917	June 7, 1917	3	+++	+
6	II	June 4, 1917	June 7, 1917	3	+++	++
7	III	June 14, 1917	June 18, 1917	4	+++	+++
8	III	June 14, 1917	June 18, 1917	4	+++	+++
9	IV	June 25, 1917	June 30, 1917	5	+++	+++
10	IV	June 25, 1917	June 30, 1917	5	+++	++
11	V	July 7, 1917	July 9, 1917	2	++	—
12	V	July 7, 1917	.....	...	—	—
13	VI	July 16, 1917	July 22, 1917	6	?	+++

TABLE 4.—RESULT OBTAINED BY THE INOCULATION OF THE VIRUS FIXE INTO THE FELINE-STRAIN BEARING RABBITS

Rabbit No.	Generation	Date of Transmission of Spirochetes	Date of Inoculation of the Virus Fixe	Interval between Transmission and Inoculation	Occurrence of Spirochetes	Lesion of the Gastric Mucosa
14	I	July 9, 1917	.....	...	—	—
15	II	July 19, 1917	July 23, 1917	4	+++	+++
16	III	July 30, 1917	Aug. 7, 1917	8	+++	+++
17	IV	Aug. 14, 1917	Aug. 20, 1917	6	+++	+++
18	IV	Aug. 14, 1917	Aug. 20, 1917	6	+++	+++
19	V	Aug. 27, 1917	Aug. 29, 1917	2	+++	++
20	V	Aug. 27, 1917	Aug. 31, 1917	4	+++	++
21	V	Aug. 27, 1917	Sept. 1, 1917	5	+++	+++
22	V	Aug. 27, 1917	Sept. 3, 1917	7	+++	+++
23	VI	Sept. 5, 1917	Sept. 6, 1917	1	+++	+
24	VII	Sept. 14, 1917	Sept. 19, 1917	5	+++	+++
25	VII	Sept. 14, 1917	Sept. 19, 1917	5	+++	+++
26	VII	Sept. 14, 1917	Sept. 19, 1917	5	+++	+++

N. B.—(1) Under "Occurrence of spirochetes": — indicates negative result; + from one to several spirochetes in a preparation; + + from one to several in several fields; + + + several or more in a field.

(2) Under "Lesion of the gastric mucosa": — indicates the apparent absence of lesions; + hyperemia and hypertrophy moderate, hemorrhage very slight, gastric contents juicy and mucus abundant; + + hyperemia and hypertrophy distinct, hemorrhage moderate and contents fluid, with only a small quantity of floating solid particles; + + + hemorrhage remarkable and contents completely fluid.

(3). Rabbits 12 and 14 are controls, which were only subjected to the inoculations of the *virus fixe*.

(4). Rabbit 13 died in the early morning on the day on which we intended to kill it; its stomach with the contents was immediately placed in the refrigerator. About ten hours later, upon examination of the stomach, no spirochetes could be detected, while marked hemorrhage was observed. This shows that, in all probability, the spirochetes were dissolved by the split products of the stomach contents.

Moreover, the stomachs of guinea-pigs previously infected with measles or scarlet fever and fed with the spirochetes constantly showed a great increase of the spirochete and the distinct hyperemia and hemorrhage of the mucosa.

The conclusion may be drawn from these results that the cases of hemorrhagic gastro-enteritis described by Balfour and Lucet are in all probability due to the secondary pathogenicity of this spirochete.

As a sequel to the foregoing experiment, we inoculated the emulsion of the gastric mucosa, containing large numbers of this spirochete, into the testes of four white rats, but no multiplication of the organism was found to have occurred.

SUMMARY

1. The stomach spirochete is an inflexible spiral with a straight longitudinal axis, the transverse section being an ellipse. It is provided with a flagellum at each end.

2. It takes stain very readily, compared with the other spirochetes, not only by the basic anilin dyes commonly used for the staining of bacteria, but by iron hematoxylin.

3. Its movement is comparatively active and very simple, progression being only forward and backward in a straight line.

4. This organism was detected in forty-three out of forty-nine dogs, in eight out of thirteen cats, in one out of thirty-eight wild rats and in every one of thirteen monkeys, but was absent in twenty rabbits, fifteen guinea-pigs, ten white rats, fifteen mice and fifteen field voles.

5. Its domicile is the stomach, especially the fundus gland.

6. It is readily soluble in saponin, sodium taurocholate and bile. It is also labile to putrefaction.

7. The introduction of salvarsan into the stomach is easily capable of sterilizing the spirochetes domiciling there.

8. The organism is readily transmitted to the stomach of the rat or mouse, but transmission to the normal rabbit or guinea-pig is very difficult.

9. If, after a certain interval, a rabbit previously infected with the spirochete be again inoculated with the *virus fixe,* the stomach of the host, at autopsy performed a week after inoculation, shows a distinct increase of spirochetes and a remarkable hemorrhagic inflammation in the mucosa. The same result was obtained in guinea-pigs previously infected with scarlet fever or measles after subsequent feeding with this spirochete.

We wish to express here our deep indebtedness to Prof. S. Kitasato, director of the Kitasato Institute, and to Profs. S. Hata and S. Kusama for their cordial guidance.

References Cited

Balfour, A. 1906.—A haemogregarine of mammals and some notes on trypanosomiasis in the Anglo-Egyptian Sudan. Jour. Trop. Med., 9: 81-92.

1906a.—Trypanosomiasis in the Anglo-Egyptian Sudan. II. Rep. of Wellcome Res. Lab. at Gordon Mem. Col. Khartoum, pp. 113-173.

Ball, V. et Roquet, M. 1911.—Spirochètes et affections hémorrhagiques gastro-intestinales du chien. Jour. Med. Vét. et Zoot., 14:257-260.

Benians, T. H. C. 1916.—Relief Staining for Bacteria and Spirochaetes. Brit. Med. Jour., 2: 722.

Bizzozero, G. 1893.—Sulla presenza di batteri nelle ghiandole rettali, e nelle ghiandole gastriche del cane. Atti R. Accad. Sci. Torino., 28: 249-251.

Carnot, P. et Lelièvre, A. 1909.—Sur la double ordination des cellules bordantes de l'estomac. Compt. rend. Soc. Biol., 66: 147-149.

1909a.—Morphologie du produit d'excrétion des cellules bordantes. Compt. rend. Soc. Biol., 66: 311-313.

Kasai, K., and Kobayashi, R. 1917.—Description of Various Species of the Spirochete Found in the Alimentary Canal of Wild Rats and Guinea-Pigs. Saikingaku Zasshi, No. 259.

Krienitz, W. 1906.—Ueber das Auftreten von Spirochaeten verschiedener Form im Mageninhalt bei Carcinoma ventriculi. Deut. med. Wchnschr., 32: 872.

1906a.—Ueber morphologische Veränderungen an Spirochaeten. Centralbl. Bakt., I, Orig., 42: 43-47.

Lucet, M. 1910.—Sur la présence de spirochètes dans un cas de gastro-entérite hémorrhagique chez le chien. Compt. rend. Acad. Sci., 151: 260-262.

1909a.—Sur les spirilles parasites des glandes gastriques du chien et du chat. Compt. rend. Soc. Biol., 66: 617-618.

Salomon, H. 1896.—Ueber das Spirillum des Säugetiermagens und sein Verhalten zu den Belegzellen. Centralbl. Bakt., I, Orig., 19: 433-442.

Suda, G. 1916.—On the Method of Staining Gland Cells in the Gastric Gland. Kyoto Igaku Zasshi., 3: 273.

Regaud, C. 1909.—Sur une curieuse localisation de spirilles parasites dans les canalisations glandulaires de la muqueuse gastrique normale, chez le chien et le chat. Compt. rend. Soc. Biol., 66: 229-231.

Description of Plate

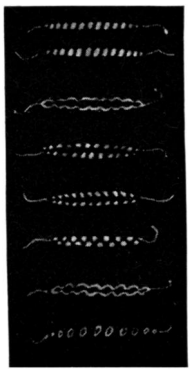
Fig. 1-3.—Three types of the spirochete.

Fig. 4.—Various forms of the spirochete under the dark-field microscope.

Fig. 5.—Various forms of the spirochete stained with Levaditi's method.

Fig. 6.—Figures of the spirochete treated with Benians' relief staining.

1



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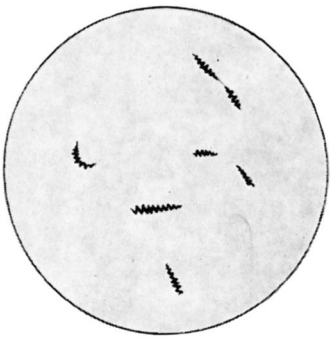

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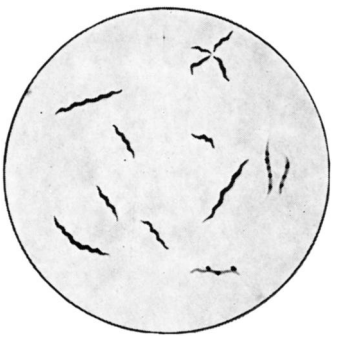

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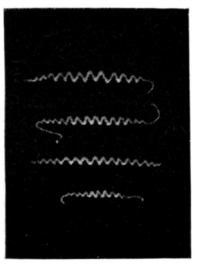

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PLATE I